Concerns about Transmission Electron Micrographs in Recent Article by Chen and Williams

This Letter to the Editor concerns a recent article published in *mBio* (1).

I am offering my comments on this article in the spirit of the correct interpretation of scientific data. My areas of expertise are in electron microscopy and in prokaryotic predators known as *Bdellovibrio* and *Bdellovibrio*-like organisms (BALOs). I have a recent article in the *International Journal of Systematic and Evolutionary Microbiology* (2), which will confirm this.

I understand the concept behind Dr. William's experiments to coinfect a marine bacterium, *Vibrio vulnificus*, with both bacteriophage and *Bacteriovorax*. However, the transmission electron micrographs in Fig. 1 of this article (1) do not support his conclusions. The cells in Fig. 1B to D that he refers to as bdelloplasts look to me like plasmolyzed *V. vulnificus* cells. The fixation method he used (3) was for encapsulated *Escherichia coli* strains and not for a marine bacterium. Most marine bacteria are fixed in the presence of salts at a concentration equivalent to that of seawater to maintain osmotic stability. This point aside, two things are usually seen inside a bdelloplast: the prey cell protoplast (with the nutrients) and the growing BALO (see the thin section in Fig. 4 in reference 3). In Fig. 1B to D of reference 1, only one structure is seen inside *V. vulnificus*. To me, this must be the protoplast of the prey cell, as there are bacteriophages there. Where is the BALO? In Fig. 1D, a growing aseptate filament of a bacterial BALO never looks like that (in good fixation conditions). There are no projections and attachments to the outer cell wall—which is why I think this looks like a plasmolyzed cell. Therefore, the legend to Fig. 1 is incorrect, in that the electron micrographs do not show a predator (*Bacteriovorax*) residing inside the prey cell.

I also have experience with scanning electron microscopy (2). The *Bacteriovorax* predators in Fig. 2 do not look well preserved. In Fig. 2A, it looks constricted in the middle. In Fig. 2B, it has an unusual shape for a vibrio—"ballooned out." Also, how do we know where the bacteriophage CK2 is on the surface of the *Vibrio* cell? There are no arrows to point them out, and what I do see on the surface look like outer membrane blebs or vesicles.

Another point that is not clear in this *mBio* article is where the agents of coinfection reside. It is an interesting concept that a predator and a bacteriophage can survive within the same cell, but would you not have to have the bacteriophage in the residual prey protoplasm and the BALO in the periplasm? Bacteriophages have very specific receptors. How would the CK2 phage be able to infect and enter the growing BALO within the periplasm? The cell wall chemistry is entirely different between the predator and prey surfaces.

With regard to the BALO used in this study—I do not understand what a "*Bacteriovorax* cluster IX" is (the *ISME Journal* article referenced is available only by payment). If the authors did isolate and cultivate a *Bacteriovorax* strain that phylogenetically is placed in cluster IX, that is fine, but an organism cannot be referred to as cluster IX. It would have to be *Bacteriovorax* sp. strain _____ (as we did for our strain JSS before we proposed a new species of *Bdellovibrio*). I am not alone in this comment, as Elio Schaechter posted in his blog "A Table for Two" in "Small Things Considered" on 14 June 2012. He said that Chen and Williams had used a BALO, "*Bdellovibrio*-like organism (actually something called cluster IX of *Bacteriovorax*) as predators." Obviously, Dr. Schaechter also did not know what a cluster IX was.

References

1. **Chen H, Williams HN.** 2012. Sharing of prey: coinfection of a bacterium by a virus and a prokaryotic predator. mBio **3**(2):e00051-12. http://dx.doi.org/10.1128/mBio.00051-12.
2. **Koval SF, et al.** 24 February 2012. *Bdellovibrio exovorus* sp. nov., a novel predator of *Caulobacter crescentus*. Int. J. Syst. Evol. Microbiol. http://dx.doi.org/10.1099/ijs.0.039701-0.
3. **Koval SF, Bayer ME.** 1997. Bacterial capsules: no barrier to *Bdellovibrio*. Microbiology **143**:749–753.

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